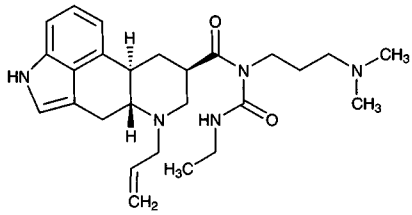
Cabergoline



Molecular formula: $C_{26}H_{37}N_5O_2$
Molecular weight: 451.61
CAS Registry No.: 81409-90-7, 85329-89-1 (diphosphate)
Merck Index: 1637

SAMPLE

Matrix: blood, urine
Sample preparation: 1 mL Plasma or urine + 0.5 (plasma) or 1 (urine) mL 500 mM pH 9 borate buffer + 2.5 mL dichloromethane:isooctane 40:60, shake on a rotary mixer for 5 min, centrifuge at 1200 g for 15 min, repeat extraction. Combine the organic layers and add them to 100 μL 100 mM phosphoric acid, vortex for 1 min, centrifuge at 1200 g for 10 min. Remove the aqueous phase and add it to 1 mL n-hexane, vortex, inject a 50 μL aliquot of the aqueous phase.

HPLC VARIABLES

Column: 250 × 4 5 μm Nucleosil C18
Mobile phase: MeCN:75 mM pH 3 phosphate buffer 20:80
Flow rate: 0.6
Injection volume: 50
Detector: E, ESA Model 5100 Coulochem, Model 5020 guard cell +670 mV, Model 5011 analytical cell, detector 1 +350 mV, detector 2 +650 mV

CHROMATOGRAM

Retention time: 13.5
Limit of quantitation: 0.30 ng/mL (urine), 0.25 ng/mL (plasma)

OTHER SUBSTANCES

Noninterfering: levodopa, methyldopa, dopamine, homovanillic acid

KEY WORDS

plasma

REFERENCE

Pianezzola,E.; Bellotti,V.; La Croix,R.; Strolin Benedetti,M. Determination of cabergoline in plasma and urine by high-performance liquid chromatography with electrochemical detection, *J.Chromatogr.*, **1992**, *574*, 170–174.

SAMPLE

Matrix: urine
Sample preparation: Add urine to Amberlite XAD-2 resin (resin/urine = 0.35-0.40), wash with water, elute with acetone, elute with acetic acid. Combine aliquots of the eluates, evaporate to dryness under vacuum, dissolve the residue in mobile phase, inject an aliquot.

HPLC VARIABLES

Guard column: Guardpak C18 (Waters)
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: Gradient. MeCN:100 mM pH 2 KCl/HCl buffer 12:88 for 5 min, to 30:70 over 15 min, maintain at 30:70 for 15 min
Flow rate: 1
Injection volume: 200
Detector: UV 280

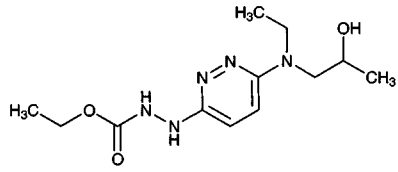
CHROMATOGRAM
Retention time: 25.4

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
rat; monkey; human; SPE

REFERENCE
Battaglia,R.; Strolin Benedetti,M.; Mantegani,S.; Castelli,M.G.; Cocchiara,G.; Dostert,P. Disposition and urinary metabolite pattern of cabergoline, a potent dopaminergic agonist, in rat, monkey and man, *Xenobiotica*, **1993**, *23*, 1377–1389.

Cadralazine



Molecular formula: $C_{12}H_{21}N_5O_3$
Molecular weight: 283.33
CAS Registry No.: 64241-34-5
Merck Index: 1669

SAMPLE

Matrix: blood
Sample preparation: 200 μL Plasma + 1.2 mL chloroform:EtOH 95:5, shake horizontally at 300 rpm for 12 min, centrifuge at 1220 g for 5 min. Remove the organic phase and add it to 100 μL 90 mM KCl in 10 mM HCl (pH 2), shake horizontally at 400 rpm for 12 min, centrifuge at 1220 g for 5 min, inject a 20 μL aliquot of the aqueous supernatant. (cf J. Chromatogr. 1984, 290, 223.)

HPLC VARIABLES

Column: 150 × 1 5 μm Nucleosil C18 in a glass-lined stainless steel column
Mobile phase: MeCN:100 mM NaH_2PO_4:1 M NaOH 18:79:3
Flow rate: 0.06
Injection volume: 20
Detector: UV 254 (2.4 μL flow cell)

CHROMATOGRAM

Retention time: 6
Internal standard: CGP 24 751
Limit of quantitation: 5 ng/mL

KEY WORDS

plasma; microbore

REFERENCE

Rouan,M.C. Microbore liquid chromatographic determination of cadralazine and cephalexin in plasma with large-volume injection, *J.Chromatogr.*, **1988**, *426*, 335–344.

SAMPLE

Matrix: blood
Sample preparation: 1 mL Whole blood + 500 μL MeCN, mix, centrifuge at 2200 g for 2 min. Remove the supernatant and saturate it with anhydrous potassium carbonate (about 2 g per 1 mL of whole blood), centrifuge at 2200 g for 3 min, remove the MeCN layer, inject a 100 μL aliquot of the MeCN layer.

HPLC VARIABLES

Guard column: 10 μm octyl (Upchurch)
Column: 150 × 4.6 3 μm C8 (Alltech)
Mobile phase: MeCN:5 mM hexanesulfonic acid in 1% acetic acid 30:70
Flow rate: 1
Injection volume: 100
Detector: UV 250

CHROMATOGRAM

Retention time: 11
Limit of quantitation: 10 ng/mL

OTHER SUBSTANCES

Noninterfering: erythromycin, cimetidine, ranitidine, aspirin, acetaminophen, creatinine, theophylline, caffeine

KEY WORDS
whole blood; salting-out

REFERENCE
Rustum,A.M. Determination of cadralazine in human whole blood using reversed-phase high-performance liquid chromatography: utilizing a salting-out extraction procedure, *J.Chromatogr.*, **1989**, *489*, 345–352.

SAMPLE
Matrix: blood, urine
Sample preparation: Plasma. 1 g Plasma + 500 μL 1 μg/mL IS in 5 mM sulfuric acid + 1 mL water + 6 mL chloroform:EtOH 95:5, shake horizontally at 300 rpm for 12 min, centrifuge at 1220 g for 5 min. Remove the organic phase and add it to 500 μL pH 2 buffer, shake horizontally at 400 rpm for 12 min, centrifuge at 1220 g for 5 min, remove the aqueous layer and inject a 100 μL aliquot. Urine. 1 g Urine + 500 μL 60 μg/mL IS in 5 mM sulfuric acid + 1 mL pH 12 phosphate buffer + 6 mL chloroform:EtOH 95:5, shake horizontally at 300 rpm for 12 min, centrifuge at 1220 g for 5 min. Remove the organic phase and add it to 500 μL pH 2 buffer, shake horizontally at 400 rpm for 12 min, centrifuge at 1220 g for 5 min, remove the aqueous layer and inject a 100 μL aliquot. (pH 2 Buffer was 90 mmoles KCl in 1 L 10 mM HCl, pH 2.06. pH 12 Buffer was 3.8 g $Na_3PO_4.12H_2O$ in 1 L water adjusted to pH 12 with about 20 mL 2 M NaOH.)

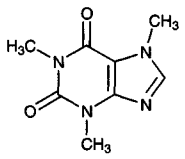
HPLC VARIABLES
Guard column: 50 × 3.2 30-40 μm Perisorb RP-8
Column: 250 × 4.6 10 μm LiChrosorb RP-8
Mobile phase: MeCN:100 mM pH 6 phosphate buffer 15:85 (Buffer was 100 mM NaH_2PO_4 adjusted to pH 6 with about 30 mL 1 M NaOH.)
Column temperature: 30
Flow rate: 2.7
Injection volume: 100
Detector: UV 254

CHROMATOGRAM
Retention time: 6
Internal standard: 2-[6-[ethyl(2-hydroxypropyl)amino]-3-pyridazinyl]hydrazine carboxylic acid propyl ester (CGP 24 751) (14)
Limit of quantitation: 10.59 nmole/g (urine), 0.141 nmole/g (plasma)

KEY WORDS
plasma; pharmacokinetics

REFERENCE
Hauffe,S.A.; Dubois,J.P. Determination of cadralazine in human plasma and urine by high-performance liquid chromatography, *J.Chromatogr.*, **1984**, *290*, 223–230.

Caffeine



Molecular formula: $C_8H_{10}N_4O_2$
Molecular weight: 194.19
CAS Registry No.: 58-08-2, 5743-12-4 (monohydrate)
Merck Index: 1674
Lednicer No.: 1 111

SAMPLE
Matrix: beverages
Sample preparation: Filter sample.

HPLC VARIABLES
Column: 150 × 4.5 5 μm Hiasil C18 (Higgins)
Mobile phase: MeOH:25 mM phosphate buffer 45:55, pH 3.0
Flow rate: 1.0
Injection volume: 20
Detector: UV 218

CHROMATOGRAM
Retention time: 2.0
Limit of detection: 1.2 mg/mL

OTHER SUBSTANCES
Simultaneous: aspartame, benzoic acid

KEY WORDS
comparison with UV spectrophotometry and capillary electrophoresis; soft drinks

REFERENCE
McDevitt,V.L.; Rodriguez,A.; Williams,K.R. Analysis of soft drinks: UV spectrophotometry, liquid chromatography, and capillary electrophoresis, *J.Chem.Educ.*, **1998**, *75*, 625–629.

SAMPLE
Matrix: beverages, blood, formulations
Sample preparation: Plasma. 100 μL Plasma + 10 μL 1,3-dimethyl-7-(2-hydroxyethyl)xanthine in water, vortex, add 1 mL acetone, vortex for 1 min, centrifuge at 2500 g for 5 min. Filter (0.45 μm) the supernatant, inject a 20 μL aliquot of the supernatant. Beverages. Dilute beverages 4-25 times with water, inject an aliquot. Tablets. Powder tablets, dissolve in water, filter, inject an aliquot of the filtrate.

HPLC VARIABLES
Column: 150 × 6 5 μm 3-aminopropylsilyl silica gel with the amino group derivatized with 1,8-naphthalic anhydride (Bunseki Kagaku 1993, 42, 817)
Mobile phase: MeOH:buffer 50:50 (Prepare buffer by dissolving 6.183 g boric acid and 1.461 g NaCl in 500 mL water, adjust pH to 6.4 with sodium borate solution.)
Column temperature: 30
Flow rate: 1
Injection volume: 20
Detector: UV 270

CHROMATOGRAM
Retention time: 24
Internal standard: 1,3-dimethyl-7-(2-hydroxyethyl)xanthine (12)
Limit of detection: 140 ng/mL

OTHER SUBSTANCES
Simultaneous: hypoxanthine, pentoxifylline, propentofylline, theobromine, theophylline, uric acid, xanthine

KEY WORDS
plasma; tablets; pharmacokinetics

REFERENCE
Nakashima,K.; Inoue,K.; Mayahara,K.; Kuroda,N.; Hamachi,Y.; Akiyama,S. Use of 3-(1,8-naphthalimido)propyl-modified silyl silica gel as a stationary phase for the high-performance liquid chromatographic separation of purine derivatives, *J.Chromatogr.A*, **1996**, *722*, 107–113.

SAMPLE
Matrix: beverages, syrup
Sample preparation: Dilute syrup ten fold. Filter (0.45 μm) beverages and diluted syrup, inject a 10-20 μL aliquot of the filtrate.

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeOH:acetic acid:water 20:5:75
Flow rate: 2
Injection volume: 10-20
Detector: UV 254

CHROMATOGRAM
Retention time: 4.5
Limit of detection: 20 ng

OTHER SUBSTANCES
Simultaneous: acesulfame, benzoic acid, dulcin, p-hydroxybenzoic acid, saccharin, vanillin

REFERENCE
Veerabhadrarao,M.; Narayan,M.S.; Kapur,O.; Sastry,C.S. Reverse phase liquid chromatographic determination of some food additives, *J.Assoc.Off.Anal.Chem.*, **1987**, *70*, 578–582.

SAMPLE
Matrix: blood
Sample preparation: Mix 100 μL serum with 50 μL 1 M sodium hydroxide, extract with 1 mL 1.5 μ g/mL IS in isopropanol:chloroform 10:90. Centrifuge at 2000 g for 1 min, decant the organic layer, dry under a gentle air flow at 60°, dissolve the residue in 0.2 mL mobile phase, inject a 50 μL aliquot.

HPLC VARIABLES
Column: 150 × 3.9 Nova Pak C18
Mobile phase: MeOH:THF:50 mM pH 4.7 ammonium acetate buffer 2:1.5:96.5
Flow rate: 1
Injection volume: 50
Detector: UV 273

CHROMATOGRAM
Internal standard: β-hydroxyethyltheophyline
Limit of quantitation: 200 ng/mL

OTHER SUBSTANCES
Extracted: theobromine, paraxanthine, theophyline

KEY WORDS
pharmakokinetics; serum

REFERENCE
Lee,T.C.; Charles,B.; Steer,P.; Flenady,V.; Shearman,A. Population pharmacokinetics of intravenous caffeine in neonates with apnea of prematurity, *Clin.Pharmacol.Ther.*, **1997**, *61*, 628–640.

SAMPLE
Matrix: blood
Sample preparation: Mix 50 μL plasma with 50 μL 20 μg/mL IS in water, vortex for 10 s, add 20 μL 20% perchloric acid, vortex for 10 s, centrifuge at 2000 g for 5 min, inject a 50 μL aliquot of the supernatant.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Spherisorb C18
Mobile phase: MeCN:THF:concentrated acetic acid:water 2:2:0.5:95.5
Column temperature: 35
Flow rate: 1
Injection volume: 50
Detector: UV 273

CHROMATOGRAM
Retention time: 7.5
Internal standard: 7-(β-hydroxypropyl)theophylline (9.2)
Limit of detection: 100 ng/mL
Limit of quantitation: 2 μg/mL

OTHER SUBSTANCES
Extracted: theophylline
Simultaneous: β-hydroxyethyltheophylline, 8-chlorotheophylline, theobromine

KEY WORDS
plasma

REFERENCE
Schreiber-Deturmeny,E.; Bruguerolle,B. Simultaneous high-performance liquid chromatographic determination of caffeine and theophylline for routine drug monitoring in human plasma, *J.Chromatogr.B*, **1996**, *677*, 305–312.

SAMPLE
Matrix: blood
Sample preparation: 200 μL Plasma + 200 μL 5 μg/mL IS, extract with 6.0 mL dichloromethane, centrifuge. Aspirate aqueous layer to waste, evaporate organic layer under a stream of nitrogen at 35°. Reconstitute residue in 200 μL MeOH:water 15:85, inject an aliquot.

HPLC VARIABLES
Column: 100 × 4.6 C8 Rainin "Short One"
Mobile phase: Gradient. MeCN:MeOH:25 mM pH 3.0 phosphate buffer 3:15:82 to 15:15:70 over 10 min.
Detector: UV 273

CHROMATOGRAM
Retention time: 4.8
Internal standard: 7-(β-chloroethyl)theophylline (9.6)

KEY WORDS
plasma

REFERENCE
Sarich,T.; Kalhorn,T.; Magee,S.; Al-sayegh,F.; Adams,S.; Slattery,J.; Goldstein,J.; Nelson,S.; Wright,J. The effect of omeprazole pretreatment on acetaminophen metabolism in rapid and slow metabolizers of S-mephenytoin, *Clin.Pharmacol.Ther.*, **1997**, *62*, 21–28.

SAMPLE
Matrix: blood
Sample preparation: Inject a 5 μL aliquot of serum directly.

HPLC VARIABLES
Column: 100 × 4.6 5-10 μm Silicalite (by sieving Silicalite, 3M Co.(?))
Mobile phase: MeCN:20 mM pH 6.9 phosphate buffer 11:89
Flow rate: 1
Injection volume: 5
Detector: UV 254

CHROMATOGRAM
Retention time: 2.99

OTHER SUBSTANCES
Also analyzed: metabolites

KEY WORDS
serum

REFERENCE
Ambrose,D.L.; Fntz,J.S. High-performance liquid chromatographic determination of drugs and metabolites in human serum and urine using direct injection and a unique molecular sieve, *J.Chromatogr.B*, **1998**, *709*, 89–96.

SAMPLE
Matrix: blood
Sample preparation: Mix 250 μL serum with 250 μL 800 mM perchloric acid, vortex, centrifuge at 14000 g for 3-4 min. Remove 350 μL supernatant, adjust to ca. pH 5.0 with 27 μL 4 M NaOH, inject an aliquot.

HPLC VARIABLES
Guard column: 10 × 4.6 Adsorbosphere ODS
Column: 250 × 4.6 5 μm Ultrasphere ODS
Mobile phase: MeOH:15 mM pH 4.9 potassium phosphate buffer 15:85 (After each elution flush the column with MeOH:water 80:20 for 5 min and then re-equilibrate with the for 5 min.)
Flow rate: 1.75
Injection volume: 100
Detector: UV 274

CHROMATOGRAM
Retention time: 17.75
Limit of quantitation: 50 ng/mL

OTHER SUBSTANCES
Extracted: metabolites
Simultaneous: acetaminophen, aspirin, hydrocortisone, prednisolone, prednisone, phenylbutazone, phenytoin

KEY WORDS
serum

REFERENCE
Holland,D.T.; Godfredsen,K.A.; Page,T.; Connor,J.D. Simple high-performance liquid chromatography method for the simultaneous determination of serum caffeine and paraxanthine following rapid sample preparation, *J.Chromatogr.B*, **1998**, *707*, 105–110.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 100 μL 0.2 mg/mL β-hydroxyethyltheophylline in buffer + 100 μL 40% aqueous trichloroacetic acid, vortex for 30 s, let stand for 5 min, centrifuge at 2000 g for 15 min, inject a 25 μL aliquot of the supernatant. (Buffer was 10 mM sodium acetate adjusted to pH 4.0 with glacial acetic acid.)

HPLC VARIABLES
Column: 10 μm μBondapak C18
Mobile phase: MeCN:buffer 6:94 (Buffer was 10 mM sodium acetate adjusted to pH 4.0 with glacial acetic acid.)
Column temperature: 40
Flow rate: 2
Injection volume: 25
Detector: UV 274

CHROMATOGRAM
Retention time: 13
Internal standard: β-hydroxyethyltheophylline (9)

OTHER SUBSTANCES
Extracted: dyphylline, theophylline, theobromine

KEY WORDS
plasma

REFERENCE
Valia,K.H.; Hartman,C.A.; Kucharczyk,N.; Sofia,R.D. Simultaneous determination of dyphylline and theophylline in human plasma by high-performance liquid chromatography, *J.Chromatogr.*, **1980**, *221*, 170–175.

SAMPLE
Matrix: blood
Sample preparation: 50 μL Serum + 50 μL 15 μg/mL β-hydroxyethyltheophylline in MeCN + 2 mL chloroform:isopropanol 95:5, mix for 30 s, centrifuge at 3000 g for 3 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 50 μL MeOH, inject a 20 μL aliquot.

HPLC VARIABLES
Column: μBondapak C18
Mobile phase: MeCN:buffer 9.75:90.25 (Buffer was 100 mM KH_2PO_4 adjusted to pH 4.0 with phosphoric acid.) (At the end of each day clean with water for 20 min and MeOH for 30 min.)
Flow rate: 2
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 8.5

Internal standard: β-hydroxyethyltheophylline (5.8)
Limit of detection: 500 ng/mL

OTHER SUBSTANCES
Extracted: dyphylline, theophylline
Simultaneous: acetaminophen, aspirin, salicylic acid, procainamide, N-acetylprocainamide
Noninterfering: benzoic acid

KEY WORDS
serum

REFERENCE
Ou,C.-N.; Frawley,V.L. Theophylline, dyphylline, caffeine, acetaminophen, salicylate, acetylsalicylate, procainamide, and N-acetylprocainamide determined in serum with a single liquid-chromatographic assay, *Clin.Chem.*, **1982**, *28*, 2157–2160.

SAMPLE
Matrix: blood
Sample preparation: 500 μL Serum or plasma + 200 μL 100 mM pH 7.0 phosphate buffer + 3 mL 0.5 μg/mL 8-chlorotheophylline in isopropanol, stir at 40000 rpm for 5 s using dental micromotor with a PTFE mixing head, centrifuge at 3500 g for 2 min. Remove the supernatant and evaporate it to dryness under a stream of nitrogen at 60°, reconstitute the residue in 50 μL MeOH, inject a 10 μL aliquot.

HPLC VARIABLES
Guard column: 50 × 3.2 30-38 μm Co:Pell ODS
Column: 250 × 4.6 5 μm Ultrasphere ODS
Mobile phase: MeCN:MeOH:10 mM pH 5.2 sodium acetate buffer 6:3:91
Column temperature: 40
Flow rate: 1.5
Injection volume: 10
Detector: UV 274

CHROMATOGRAM
Retention time: 12.1
Internal standard: 8-chlorotheophylline (8.85)

OTHER SUBSTANCES
Extracted: dyphylline, theophylline, proxyphylline, paraxanthine
Simultaneous: cefoxitin
Noninterfering: carbenicillin, cefoperazone, cephacetril, heparin, penicillin G, phenytoin, phenobarbital

KEY WORDS
plasma; serum; pharmacokinetics

REFERENCE
Wenk,M.; Eggs,B.; Follath,F. Simultaneous determination of diprophylline, proxyphylline and theophylline in serum by reversed-phase high-performance liquid chromatography, *J.Chromatogr.*, **1983**, *276*, 341–348.

SAMPLE
Matrix: blood
Sample preparation: 200 μL Serum + 200 μL 4 μg/mL 8-chlorotheophylline in MeCN, mix, centrifuge, evaporate the supernatant to dryness, reconstitute in 400 μL mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 100 × 3 8 μm octadecyl CP-tm-Spher C18 glass column (Chrompack)
Mobile phase: MeCN:20 mM sodium acetate 20:80 adjusted to pH 4.4 with phosphoric acid
Flow rate: 0.8
Injection volume: 20
Detector: UV 273

CHROMATOGRAM
Retention time: 10.0
Internal standard: 8-chlorotheophylline (8.8)
Limit of detection: 1000 ng/mL

OTHER SUBSTANCES
Simultaneous: theophylline

KEY WORDS
serum

REFERENCE
Van Damme,M.; Molle,L.; Abi Khalil,F. Useful sample handlings for reversed phase high performance liquid chromatography in emergency toxicology, *J.Toxicol.Clin.Toxicol.*, **1985**, *23*, 589–614.

SAMPLE
Matrix: blood
Sample preparation: 100 μL Serum + 1 mL reagent, vortex for 10 min, centrifuge for 2-3 min. Remove the lower organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 200 μL mobile phase, inject a 40 μL aliquot. (Reagent was 1.5 mg β-hydroxyethyltheophylline and 25 μL glacial acetic acid in 100 mL chloroform:isopropanol 95:5.)

HPLC VARIABLES
Guard column: 30 mm long 5 μm Ultrasphere ion-pair
Column: 150 × 4.6 5 μm Ultrasphere ion-pair
Mobile phase: MeCN:MeOH:water:1 M tetra-n-butylammonium hydroxide 3.5:3.5:91:2 containing 1.82 g Trizma base (Tris, tris(hydroxymethyl)aminomethane), pH adjusted to 7.50 ± 0.03 with concentrated HCl
Flow rate: 1.2
Injection volume: 40
Detector: UV 280

CHROMATOGRAM
Retention time: k' 6.3
Internal standard: β-hydroxyethyltheophylline (k' 4.2)

OTHER SUBSTANCES
Extracted: theophylline, theobromine, 1,7-dimethyl xanthine
Simultaneous: acetaminophen, acetazolamide, allopurinol, dimethylurea, dyphylline, 3-methylxanthine, oxypurinol, procainamide, sulfadiazine, sulfamethazine, uric acid
Noninterfering: ampicillin, cefazolin, cephalothin, cephapirin, chlorotheophylline, 1,3-dimethyluric acid, gentamicin, lidocaine, methicillin, methylurea, 3-methyluric acid, quinidine, sulfamerazine, 1,3,7-trimethyluric acid

KEY WORDS
serum

REFERENCE
Lauff,J.J. Ion-pair high-performance liquid chromatographic procedure for the quantitative analysis of theophylline in serum samples, *J.Chromatogr.*, **1987**, *417*, 99–109.

SAMPLE

Matrix: blood

Sample preparation: 100 μL Serum + 100 μL buffer + 1.5 mL IS in 5% isopropanol in chloroform, vortex for 30 s, centrifuge. Remove the organic layer and evaporate it to dryness under a stream of air at room temperature, reconstitute the residue in 100 μL mobile phase, inject a 6-10 μL aliquot. (Buffer was 13.6 g KH_2PO_4 in 90 mL water, pH adjusted to 6.8 with about 3 mL 10 M NaOH, made up to 100 mL.)

HPLC VARIABLES

Guard column: 20 × 4.6 Supelguard LC-1 (Supelco)
Column: 250 × 4.6 5 μm Supelcosil LC-1 (Supelco)
Mobile phase: MeOH:MeCN:buffer 17.5:17.5:65 (Buffer was 2.72 g KH_2PO_4 in 1.9 L water, pH adjusted to 6.3 with about 2 mL 1 M NaOH, made up to 2 L.)
Flow rate: 2
Injection volume: 6-10
Detector: UV 273

CHROMATOGRAM

Retention time: 2.18
Internal standard: 3-isobutyl-1-methylxanthine (3.15)

OTHER SUBSTANCES

Extracted: acetaminophen, amobarbital, barbital, carbamazepine, chloramphenicol, ethosuximide, mephobarbital, methsuximide, pentobarbital, phenobarbital, phenytoin, primidone, secobarbital, theophylline, thiopental

Also analyzed: acetanilide, N-acetylcysteine, N-acetylprocainamide, ampicillin, aspirin, butabarbital, butalbital, chlorpropamide, cimetidine, codeine, cyheptamide, diazoxide, diflunisal, diphylline, disopyramide, ethchlorvynol, gentisic acid, glutethimide, heptabarbital, hexobarbital, ibuprofen, indomethacin, ketoprofen, mefenamic acid, mephenytoin, methaqualone, methsuximide, methyl salicylate, methyprylon, morphine, naproxen, nirvanol, oxphenylbutazone, phenacetin, phensuximide, phenylbutazone, procainamide, salicylamide, salicylic acid, sulfamethoxazole, sulindac, tolmetin, trimethoprim, vancomycin

Noninterfering: amikacin, gentamicin, meprobamate, netilmicin, quinidine, tetracycline, tobramycin, valproic acid

KEY WORDS

serum

REFERENCE

Meatherall,R.; Ford,D. Isocratic liquid chromatographic determination of theophylline, acetaminophen, chloramphenicol, caffeine, anticonvulsants, and barbiturates in serum, *Ther.Drug Monit.*, **1988**, *10*, 101–115.

SAMPLE

Matrix: blood

Sample preparation: Prepare an SPE cartridge by plugging the end of a 1 mL disposable pipette tip with glass wool and adding about 100 mg Chromosorb P/NAW. Add 50 μL plasma then 50 μL 10 μg/mL tolylphenobarbital in 200 mM HCl to the SPE cartridge, let stand for 2 min, elute with 1 mL chloroform:isopropanol 6:1. Evaporate the eluate to dryness under a stream of nitrogen at 30°, reconstitute the residue in 100 μL mobile phase, inject a 15 μL aliquot.

HPLC VARIABLES

Column: 150 × 4.6 5 μm Supelcosil-LC-8
Mobile phase: MeCN:water 20:80
Flow rate: 3.3
Injection volume: 15

Detector: UV 208

CHROMATOGRAM

Retention time: 0.93
Internal standard: tolylphenobarbital (7.57)
Limit of detection: 50-100 ng/mL

OTHER SUBSTANCES

Extracted: theophylline, barbital, ethosuximide, primidone, carbamazepinediol, phenacemide, methyprylon, nirvanol, phenobarbital, chloramphenicol, butabarbital, carbamazepine epoxide, mephenytoin, pentobarbital, amobarbital, carbamazepine, glutethimide, phenytoin, secobarbital, methaqualone
Noninterfering: acetaminophen, amikacin, amitriptyline, clonazepam, cyclosporine, desipramine, diazepam, digoxin, disopyramide, gentamicin, imipramine, lidocaine, methotrexate, N-acetylprocainamide, netilmicin, nortriptyline, procainamide, quinidine, salicylic acid, sulfamethoxazole, tobramycin, trimethoprim, valproic acid, p-hydroxyphenobarbital, vancomycin

KEY WORDS

plasma; SPE

REFERENCE

Svinarov,D.A.; Dotchev,D.C. Simultaneous liquid-chromatographic determination of some bronchodilators, anticonvulsants, chloramphenicol, and hypnotic agents, with Chromosorb P columns used for sample preparation, *Clin.Chem.*, **1989**, *35*, 1615–1618.

SAMPLE

Matrix: blood
Sample preparation: Inject 20 μL serum onto column A with mobile phase A and elute to waste, after 1 min backflush the contents of column A onto column B with mobile phase B, after 1 min remove column A from the circuit, elute column B with mobile phase B, monitor the effluent from column B. Re-equilibrate column A with mobile phase A.

HPLC VARIABLES

Column: A 30 × 4.6 IRSP silica (for preparation see Anal. Chem. 1989, 61, 2445); B 150 × 4.6 TSK gel ODS-80TM
Mobile phase: A 20 mM NaH_2PO_4; B MeCN:100 mM NaH_2PO_4 10:90
Flow rate: A 0.8; B 1
Injection volume: 20
Detector: UV 275

CHROMATOGRAM

Retention time: 12.5
Limit of quantitation: 1 μg/mL

OTHER SUBSTANCES

Extracted: theobromine, theophylline

KEY WORDS

serum; column-switching

REFERENCE

Haginaka,J.; Wakai,J.; Yasuda,H.; Kimura,Y. Determination of anticonvulsant drugs and methyl xanthine derivatives in serum by liquid chromatography with direct injection: column-switching method using a new internal-surface reversed-phase silica support as a precolumn, *J.Chromatogr.*, **1990**, *529*, 455–461.

SAMPLE
Matrix: blood
Sample preparation: 50 μL Serum + 10 μL 25 μg/mL lidocaine in 4 mM HCl, mix, add 100 μL 1 M pH 9.0 borate buffer, add 1 mL chloroform:EtOH 82.5:17.5, mix, centrifuge. Remove the organic layer and evaporate it to dryness, reconstitute the residue in 50 μL mobile phase, inject an aliquot.

HPLC VARIABLES
Column: 300 × 2 10 μm μBondapak C18
Mobile phase: MeOH:MeCN:buffer 12:16:72 (Buffer was 31 mM sodium acetate adjusted to pH 5.1 with 40% phosphoric acid containing 0.15 mM tetrabutylammonium phosphate.)
Flow rate: 0.3
Injection volume: 20
Detector: UV 230

CHROMATOGRAM
Retention time: 5
Internal standard: lidocaine (10)
Limit of detection: 12.5 ng/mL

OTHER SUBSTANCES
Extracted: cocaine
Simultaneous: barbital, phenobarbital, flumazepil, mazindol, hexobarbital
Noninterfering: amphetamine, desipramine, tetracaine, methadone, reserpine, buspirone, diazepam, haloperidol, chlordiazepoxide, oxazepam, midazolam, clonazepam, chlorpromazine, pentobarbital
Interfering: nicotine, procaine, cotinine

KEY WORDS
serum; rat

REFERENCE
Lau,C.E.; Ma,F.; Falk,J.L. Simultaneous determination of cocaine and its metabolites with caffeine in rat serum microsamples by high-performance liquid chromatography, *J.Chromatogr.*, **1990**, *532*, 95–103.

SAMPLE
Matrix: blood
Sample preparation: 100 μL Plasma or serum + 35 μL 22 μg/mL 3-ethylxanthine in 20 mM pH 4.0 acetate buffer, add to a Celute-MX SPE cartridge (Jones Chromatography), let stand for 10 min, elute with two portions of isopropanol:dichloromethane 10:90, evaporate the eluate to dryness under a stream of nitrogen below 37°, reconstitute the residue in 100 μL mobile phase B, centrifuge at 4400 rpm in a refrigerated centrifuge for 5 min, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.5 3 μm ODS Apex I (Jones Chromatography)
Mobile phase: Gradient. A was MeCN:THF:10 mM pH 4.0 acetate buffer 25:2:73. B was THF:10 mM pH 4.0 acetate buffer 0.01:99.99. From A:B 0:100 increasing at 2.1% A/min.
Column temperature: 50
Flow rate: 0.8
Injection volume: 20
Detector: UV 273

CHROMATOGRAM
Retention time: 20.17
Internal standard: 3-ethylxanthine (13.64)

OTHER SUBSTANCES
Simultaneous: theophylline, theobromine, paraxanthine, methylxanthines, dimethyluric acids, trimethyluric acid, acetaminophen

KEY WORDS
plasma; serum; SPE

REFERENCE
Leakey,T.E. Simultaneous analysis of theophylline, caffeine and eight of their metabolic products in human plasma by gradient high-performance liquid chromatography, *J.Chromatogr.*, **1990**, *507*, 199–220.

SAMPLE
Matrix: blood
Sample preparation: 100 μL Serum + 500 μL 220 μg/mL 4-nitroacetanilide in ethyl acetate, vortex for 30 s, centrifuge at 7000 g for 1 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 100 μL mobile phase, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 33 × 4.6 3 μm C18 (Perkin-Elmer)
Mobile phase: Isopropanol:100 mM pH 5.0 sodium acetate buffer 2:98
Flow rate: 2
Injection volume: 10
Detector: UV 278

CHROMATOGRAM
Retention time: 3.85
Internal standard: 4-nitroacetanilide (8.27)
Limit of quantitation: 10000 ng/mL

OTHER SUBSTANCES
Extracted: chloramphenicol
Simultaneous: theobromine, theophylline, diphylline, chloramphenicol 3-monosuccinate
Noninterfering: acetaminophen, N-acetylprocainamide, amikacin, amitriptyline, carbamazepine, cyclosporine, digoxin, desipramine, disopyramide, ethosuximide, gentamicin, imipramine, lidocaine, lithium, methotrexate, netilmicin, nortriptyline, phenobarbital, phenytoin, primidone, procainamide, quinidine, salicylic acid, theophylline, tobramycin, valproic acid, vancomycin

KEY WORDS
serum

REFERENCE
Markin,R.S.; Wadman,M.C.; Bottjen,P.L.; Haven,M.C.; Huth,J.A. Short-column liquid chromatographic assay for caffeine and chloramphenicol in serum, *J.Chromatogr.*, **1990**, *525*, 464–470.

SAMPLE
Matrix: blood
Sample preparation: Centrifuge, filter (0.45 μm), inject an aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm internal surface reversed phase Pinkerton, silica derivatized with glycine-phenylalanine-phenylalanine (Regis) (periodically reverse the column)
Mobile phase: 100 mM pH 6.8 phosphate buffer
Flow rate: 0.3
Injection volume: 10

Detector: UV 275

CHROMATOGRAM
Retention time: 13.03
Limit of detection: <1000 ng/mL

OTHER SUBSTANCES
Extracted: dyphylline, doxofylline, theophylline
Noninterfering: acetaminophen, amitriptyline, amphetamine, atropine, benzoylecgonine, benztropine, caffeine, carbamazepine, carisoprodol, chlorpheniramine, chlorpromazine, chlorprothixene, cimetidine, cocaine, codeine, dextromethorphan, diazepam, diphenhydramine, diphenoxilate, disopyramide, doxepin, doxylamine, emetine, erythromycin, flurazepam, glutethimide, hydrocortisone, hydromorphone, hydroxyzine, imipramine, lidocaine, loxapine, meperidine, meprobamate, methadone, methamphetamine, methapyrilene, methaqualone, methocarbamol, methylphenidate, nicotine, nordiazepam, nortriptyline, orphenadrine, papaverine, pentazocine, phenacetin, phencyclidine, phenmetrazine, phenolphthalein, phentermine, phenylpropanolamine, phenytoin, prazepam, procainamide, procaine, propoxyphene, propranolol, protriptyline, pseudoephedrine, pyrilamine, quinine, salicylamide, spironolactone, strychnine, terpin hydrate, thioridazine, thiothixene, triamterene, trifluoperazine, triflupromazine, trihexyphenidyl, trimeprazine, trimethobenzamide, trimethoprim, tripelennamine

KEY WORDS
plasma; serum; direct injection

REFERENCE
Tagliaro,F.; Dorizzi,R.; Frigerio,A.; Marigo,M. Non-extraction HPLC method for simultaneous measurement of dyphylline and doxofylline in serum, *Clin.Chem.*, **1990**, *36*, 113–115.

SAMPLE
Matrix: blood
Sample preparation: 10 µL Plasma + 300 µL 100 mM pH 6.0 KH_2PO_4 buffer + 100 µL 10 µg/mL diprophylline + 2 mL chloroform:isopropanol 50:50, vortex for 30 s, centrifuge at 2000 rpm for 10 min. Remove the organic layer and evaporate it to dryness under a stream of air at 40°, reconstitute the residue in 100 µL mobile phase, inject a 25 µL aliquot.

HPLC VARIABLES
Column: NovaPak C18 radial compression
Mobile phase: MeOH:MeCN:10 mM KH_2PO_4 9:2.5:90
Flow rate: 2
Injection volume: 25
Detector: UV 270

CHROMATOGRAM
Retention time: 17
Internal standard: diprophylline (10)

OTHER SUBSTANCES
Extracted: theophylline, 3-methylxanthine, theobromine

KEY WORDS
plasma

REFERENCE
Augustijns,P.; Verbeke,N. A microassay method for the determination of theophylline in biological samples using HPLC with electrochemical detection, *J.Liq.Chromatogr.*, **1992**, *15*, 1303–1313.

SAMPLE
Matrix: blood
Sample preparation: 100 μL Serum + 100 μL 20 μg/mL hydroxyethyltheophylline in 2 M perchloric acid, vortex, centrifuge 5 min, inject 50 μL aliquot of supernatant.

HPLC VARIABLES
Column: 125 × 4 LiChroSpher RP-8 5 μm
Mobile phase: MeOH:buffer 15:85 (Buffer was 5 mL 2 M sodium acetate + 845 mL water, pH adjusted to 4.0 with acetic acid.)
Column temperature: 45
Flow rate: 1.5
Injection volume: 50
Detector: UV 282

CHROMATOGRAM
Retention time: 9.6
Internal standard: hydroxyethyltheophylline (5.6)

OTHER SUBSTANCES
Simultaneous: theophylline, chloramphenicol

KEY WORDS
serum

REFERENCE
Hannak,D.; Haux,P.; Scharbert,F.; Kattermann,R. Liquid chromatographic analysis of phenobarbital, phenytoin, and theophylline, *Wien.Klin.Wochenschr.Suppl.*, **1992**, *191*, 27–31.

SAMPLE
Matrix: blood
Sample preparation: 500 μL Plasma + 50 μL 3 μg/mL 8-chlorotheophylline in mobile phase + 100 μL 1 M HCl + 3 mL dichloromethane, vortex for 2 min, centrifuge at 1200 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue in 100 μL mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm TSK gel ODS-80TM (Tosoh)
Mobile phase: MeOH:100 mM NaH_2PO_4 30:70
Flow rate: 0.8
Injection volume: 20
Detector: UV 274

CHROMATOGRAM
Retention time: 8
Internal standard: 8-chlorotheophylline (11.7)
Limit of quantitation: 5 ng/mL

OTHER SUBSTANCES
Extracted: theophylline, theobromine, paraxanthine, metabolites
Noninterfering: acetaminophen, phenylbutazone, phenacetin, thiamine, salicylic acid, phenobarbital, chlorpheniramine, trimethadione, hydrocortisone, prednisolone, prednisone, phenytoin, aspirin, ethenzamide

KEY WORDS
plasma

REFERENCE

Tanaka,E. Simultaneous determination of caffeine and its primary demethylated metabolites in human plasma by high-performance liquid chromatography, *J.Chromatogr.*, **1992**, *575*, 311–314.

SAMPLE

Matrix: blood

Sample preparation: Dilute serum with an equal volume 7.5 μg/mL theobromine, inject a 20 μL aliquot directly.

HPLC VARIABLES

Column: 150 × 4.6 ChromSpher 5 BioMatrix (Chrompack)
Mobile phase: MeCN:water 5:95
Flow rate: 1
Injection volume: 20
Detector: UV 280

CHROMATOGRAM

Retention time: 4.2
Internal standard: theobromine (3.3)

OTHER SUBSTANCES

Simultaneous: theophylline

KEY WORDS

serum

REFERENCE

Helmsing,P.J.; Huisman,R.; van der Weele,A. HPLC determination of caffeine and theophylline by direct serum injection [letter], *Clin.Chem.*, **1993**, *39*, 1348–1349.

SAMPLE

Matrix: blood

Sample preparation: Condition an Merck Extrelut-3 glass extraction column with 12 mL dichloromethane the day before. 1.5 mL Serum + 100 μL 3 μg/mL N-ethylnorcotinine in water + 1.4 mL 0.5 M NaOH. Add to column, after 15 min elute under gravity with dichloromethane:isopropanol 9:1. Add 300 μL 25 mM HCl in MeOH to the organic phase and evaporate it to dryness under nitrogen. Redissolve in 100 μL water and inject a 20 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 μm Supelcosil LC8DB

Mobile phase: Gradient. A was MeCN:water 3.6:96.4 containing 2 mL/L triethylamine, 12 mM sodium heptanesulfonate, 12 mM K_2HPO_4, 12 mM citric acid adjusted to pH 4.7 with citric acid. B was MeCN:water 19.7:80.3 containing 2 mL/L triethylamine, 12 mM sodium heptanesulfonate, 12 mM K_2HPO_4, 12 mM citric acid adjusted to pH 5.2 with citric acid. A:B 100:0 for 15 min then to 50:50 over 20 min using a concave gradient. Re-equilibrate for 15 min before next injection.

Flow rate: 1.5
Injection volume: 20
Detector: UV 254

CHROMATOGRAM

Retention time: 27
Internal standard: N-ethylnorcotinine (33)

OTHER SUBSTANCES

Simultaneous: nicotine, cotinine-N-oxide, trans-3'-hydroxycotinine, norcotinine, cotinine

KEY WORDS
serum

REFERENCE
Zuccaro,P.; Altieri,I.; Rosa,M.; Passa,A.R.; Pichini,S.; Ricciarello,G.; Pacifici,R. Determination of nicotine and four metabolites in the serum of smokers by high-performance liquid chromatography with ultraviolet detection, *J.Chromatogr.*, **1993**, *621*, 257–261.

SAMPLE
Matrix: blood
Sample preparation: Rock 5 mL whole blood + 10 mL water + 8.5 mL Na_2WO_4 in a 50 mL stoppered tube for 1 min, add 6 mL $NiCl_2$, rock for 5 min, add 15 mL dichloromethane: isobutyl alcohol:THF 30:45:25, centrifuge at 2500 g for 15 min. Remove organic phase and repeat the process. Filter all organic phases through a 40-90 μm filter and evaporate to dryness in a 100 mL porcelain dish at a moderate temperature in a sand bath. Take up residue in 500 μL MeCN:water 80:20, inject a 20 μL aliquot. (Na_2WO_4 prepared by mixing 10 g $Na_2WO_4.2H_2O$ in 38 mL of 2 M NaOH and 2.5 g of $NaHCO_3$ and making up to 100 mL. $NiCl_2$ was 17% w/v $NiCl_2$ in water.)

HPLC VARIABLES
Column: 200 × 4.6 5 μm Hypersil C8
Mobile phase: A = MeCN; B = 20 mM n-propylamine adjusted to pH 5 with 85% phosphoric acid. A:B from 15:85 to 20:80 over 5 min to 45:55 over another 15 min to 65:35 over another 5 min
Injection volume: 20
Detector: UV 230

CHROMATOGRAM
Retention time: 5
Limit of detection: 0.10 ppm

OTHER SUBSTANCES
Extracted: buprenorphine, cocaine, codeine, diamorphine, ethylmorphine, lidocaine, methaqualone, morphine, naloxone, noscapine, papaverine, pentazocine, procaine
Also analyzed: bromazepam, clonazepam, diazepam, flunitrazepam, flurazepam, medazepam, nitrazepam, oxazepam

KEY WORDS
whole blood

REFERENCE
Bernal,J.L.; Del Nozal,M.J.; Rosas,V.; Villarino,A. Extraction of basic drugs from whole blood and determination by high performance liquid chromatography, *Chromatographia*, **1994**, *38*, 617–623.

SAMPLE
Matrix: blood
Sample preparation: Wash PCPure cartridge containing 0.4 g hydroxyapatite with 10 mL MecN and remove MeCN by evaporation. 75 μL Plasma + 25 μL of 50 μg/mL IS in 0.5% aqueous MeCN injected onto PCPure cartridge, elute with MeCN:water 10:90. Use first 600 μL of eluate, inject 20 μL aliquots.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Inertsil ODS-2
Mobile phase: MeCN:water 5:95
Column temperature: 40
Flow rate: 1
Injection volume: 20

Detector: UV 280

CHROMATOGRAM
Retention time: 15
Internal standard: 7-(2-hydroxyethyl)theophylline (10)

OTHER SUBSTANCES
Simultaneous: theophylline

KEY WORDS
plasma; SPE

REFERENCE
Iwase,H.; Gondo,K.; Koike,T.; Ono,I. Novel precolumn deproteinization method using a hydroxyapatite cartridge for the determination of theophylline and diazepam in human plasma by high-performance liquid chromatography with ultraviolet detection, *J.Chromatogr.B*, **1994**, *655*, 73–81.

SAMPLE
Matrix: blood
Sample preparation: 100 μL Plasma + 100 μL 20 μg/mL theophylline + 8 mL dichloromethane, shake for 20 min, centrifuge at 2500 rpm for 20 min. Remove 7 mL of the organic layer and evaporate it to dryness under nitrogen or at 60°. Dissolve residue in 200 μL mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 150 × 6 Shimpack CLS-ODS (Shimadzu)
Mobile phase: MeCN:0.5 mM phosphoric acid 10:90
Column temperature: 40
Flow rate: 1.5
Injection volume: 20
Detector: UV 273

CHROMATOGRAM
Internal standard: theophylline

KEY WORDS
plasma; rat

REFERENCE
Lee,C.K.; Uchida,T.; Kitagawa,K.; Yagi,A.; Kim,N.-S.; Goto,S. Skin permeability of various drugs with different lipophilicity, *J.Pharm.Sci.*, **1994**, *83*, 562–565.

SAMPLE
Matrix: blood
Sample preparation: 250 μL Plasma + 25 μL 100 μg/mL theophylline, extract with chloroform:isopropanol 85:15. Remove the organic layer and evaporate it to dryness, reconstitute the residue in mobile phase, inject an aliquot.

HPLC VARIABLES
Column: 100 × 4.6 microsphere C18 (Chrompack)
Mobile phase: MeCN:55 mM pH 4.0 sodium acetate buffer 6:94
Flow rate: 1.2
Detector: UV 278

CHROMATOGRAM
Internal standard: theophylline

KEY WORDS
plasma; pig; pharmacokinetics

REFERENCE
Monshouwer,M.; Witkamp,R.F.; Nijmeijer,S.M.; Pijpers,A.; Verheijden,J.H.M.; Van Miert,A.S.J.P.A.M. Selective effects of a bacterial infection (*Actinobacillus pleuropneumoniae*) on the hepatic clearance of caffeine, antipyrine, paracetamol, and indocyanine green in the pig, *Xenobiotica*, **1995**, *25*, 491–499.

SAMPLE
Matrix: blood
Sample preparation: Filter (0.22 μm), inject a 20 μL aliquot of the filtrate.

HPLC VARIABLES
Column: 250 × 4 5 μm LiChrospher 100 Diol
Mobile phase: MeCN:50 mM pH 6.9 phosphate buffer 1.8:98.2
Flow rate: 0.6
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 6

OTHER SUBSTANCES
Extracted: theophylline

KEY WORDS
serum; direct injection

REFERENCE
Nimura,N.; Itoh,H.; Kinoshita,T. Diol-bonded silica gel as a restricted access packing forming a binary-layered phase for direct injection of serum for the determination of drugs, *J.Chromatogr.A*, **1995**, *689*, 203–210.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Serum + 100 μL 70% perchloric acid, centrifuge at 2000 g for 10 min. Remove the supernatant and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue in 100 μL water, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Supelcosil LC8DB
Mobile phase: MeCN:MeOH:THF:buffer 4.3:2.3:0.3:93.1 (Buffer was 2 mL/L triethylamine containing 12 mM sodium heptanesulfonate, 12 mM K_2HPO_4, and 12 mM citric acid, adjusted to pH 4.7 with citric acid.)
Flow rate: 1.4
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 11
Limit of detection: 5 ng/mL

OTHER SUBSTANCES
Extracted: nicotine, cotinine

KEY WORDS
serum

REFERENCE
Pichini,S.; Altieri,I.; Passa,A.R.; Rosa,M.; Zuccaro,P.; Pacifici,R. Use of solvent optimization software for rapid selection of conditions for reversed-phase high-performance liquid chromatography of nicotine and its metabolites, *J.Chromatogr.A*, **1995**, *697*, 383–388.

SAMPLE
Matrix: blood
Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform: isopropanol:n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 μL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 μL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES
Column: 300 × 3.9 4 μm NovaPack C18
Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)
Column temperature: 30
Flow rate: 0.8
Injection volume: 50
Detector: UV 273

CHROMATOGRAM
Retention time: 3.06
Limit of detection: <120 ng/mL

KEY WORDS
whole blood; plasma; interferences may occur—compounds(all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; acepromazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; tri-

fluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

SAMPLE

Matrix: blood, CSF

Sample preparation: 200 μL Serum, plasma, or CSF + 300 μL reagent. Flush column A to waste with 500 μL 500 mM ammonium sulfate, inject sample onto column A, flush column A to waste with 500 μL 500 mM ammonium sulfate, elute the contents of column A onto column B with mobile phase, monitor the effluent from column B. (Reagent was 8.05 M guanidine hydrochloride and 1.02 M ammonium sulfate in water.)

HPLC VARIABLES

Column: A 30 × 2.1 40 μm preparative grade C18 (Analytichem); B 250 × 4.6 10 μm Partisil C8

Mobile phase: Gradient. A was 50 mM pH 4.5 KH_2PO_4. B was MeCN:isopropanol 80:20. A:B 90:10 for 1 min, to 30:70 over 15 min, maintain at 30:70 for 4 min.

Column temperature: 50

Flow rate: 1.5

Detector: UV 280 for 5 min then UV 254

CHROMATOGRAM

Retention time: 6.16

Internal standard: heptanophenone (19.2)

OTHER SUBSTANCES

Extracted: acetazolamide, ampicillin, bromazepam, carbamazepine, chloramphenicol, chlorothiazide, diazepam, droperidol, ethionamide, furosemide, isoniazid, methadone, penicillin G, phenobarbital, phenytoin, prazepam, propoxyphene, pyrazinamide, rifampin, trimeprazine, trimethoprim

KEY WORDS

plasma; serum; column-switching

REFERENCE

Seifart,H.I.; Kruger,P.B.; Parkin,D.P.; van Jaarsveld,P.P.; Donald,P.R. Therapeutic monitoring of antituberculosis drugs by direct in-line extraction on a high-performance liquid chromatography system, *J.Chromatogr.*, **1993**, *619*, 285–290.

SAMPLE

Matrix: blood, CSF, gastric contents, urine

Sample preparation: 200 μL Serum, urine, CSF, or gastric fluid + 300 μL reagent. Flush column A to waste with 500 μL 500 mM ammonium sulfate, inject sample onto column A, flush column A to waste with 500 μL 500 mM ammonium sulfate, backflush the contents of column A onto column B with mobile phase, monitor the effluent from column B. (Reagent was 8.05 M guanidine HCl and 1.02 M ammonium sulfate in water.)

HPLC VARIABLES

Column: A 40 μm preparative grade C18 (Analytichem); B 75 × 2.1 pellicular C18 (Whatman) + 250 × 4.6 5 μm C8 end-capped (Whatman)
Mobile phase: Gradient. A was 50 mM pH 4.5 KH_2PO_4. B was MeCN:isopropanol 80:20. A:B 90:10 for 1 min, to 30:70 over 20 min.
Column temperature: 50
Flow rate: 1.5
Detector: UV 220

CHROMATOGRAM

Retention time: 6.1
Internal standard: heptanophenone (19)

OTHER SUBSTANCES

Extracted: acetaminophen, allobarbital, azinphos, barbital, brallobarbitone, bromazepam, butethal, carbamazepine, carbaryl, cephaloridine, chloramphenicol, chlordiazepoxide, chlorothiazide, chlorvinphos, clothiapine, cocaine, coomassie blue, desipramine, diazepam, diphenhydramine, dipipanone, ethylbromphos, flufenamic acid, formothion, griseofulvin, indomethacin, lidocaine, lorazepam, malathion, medazepam, midazolam, oxazepam, paraoxon, penicillin G, pentobarbital, prazepam, propoxyphene, prothiophos, quinine, salicylic acid, secobarbital, strychnine, sulfamethoxazole, theophylline, thiopental, thioridazine, trimethoprim

KEY WORDS

serum; column-switching

REFERENCE

Kruger,P.B.; Albrecht,C.F.De V.; Jaarsveld,P.P. Use of guanidine hydrochloride and ammonium sulfate in comprehensive in-line sorption enrichment of xenobiotics in biological fluids by high-performance liquid chromatography, *J.Chromatogr.*, **1993**, *612*, 191–198.

SAMPLE

Matrix: blood, gastric contents, pancreatic juice
Sample preparation: 500 μL Serum, pancreatic juice, or gastric juice + 25 μL 200 μg/mL β-hydroxyethyltheophylline in water+ 500 μL MeCN, centrifuge at 6000 rpm for 10 min, remove supernatant and add it to 1.8 mL chloroform, vortex, centrifuge at 6000 rpm for 10 min. Remove the lower organic phase and evaporate it to dryness at 60° under a stream of air, dissolve the residue in 500 μL mobile phase, inject a 50 μL aliquot.

HPLC VARIABLES

Guard column: C18 LichroCART
Column: 250 × 4.6 7 μm Lichrosorb C18
Mobile phase: THF:MeOH:10 mM pH 3.5 KH_2PO_4 1:20:79
Flow rate: 0.8
Injection volume: 50
Detector: UV 280

CHROMATOGRAM

Retention time: 10.78
Internal standard: β-hydroxyethyltheophylline (7.48)
Limit of quantitation: 250 ng/mL

OTHER SUBSTANCES

Simultaneous: theophylline, 1-methyluric acid, 1-methylxanthine, theobromine, paraxanthine

KEY WORDS

serum; dog

REFERENCE
Casoli,P.; Vérine,H. High performance liquid chromatographic determination of methylxanthines in canine serum, gastric and pancreatic juices, *Biomed.Chromatogr.*, **1990**, *4*, 209–213.

SAMPLE
Matrix: blood, milk
Sample preparation: 25 μL serum + 100 μL 250 ng/mL β-hydroxyethyltheophylline in water + 200 μL 200 mM pH 6.0 phosphate buffer + 3 mL dichloromethane, shake at 120 oscillations/min for 20 min, centrifuge at 174 g. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 60°, reconstitute the residue in 230 μL water, heat at 90° for 6 min, vortex, inject a 180 μL aliquot. (No details given for milk extraction.)

HPLC VARIABLES
Guard column: Corasil Bondapak C18
Column: 5 μm radial-compression C18 (Waters)
Mobile phase: MeOH:THF:10 mM KH_2PO_4 9:1:90 adjusted to pH 3.5
Flow rate: 1.2
Injection volume: 180
Detector: UV 214

CHROMATOGRAM
Retention time: 16.5
Internal standard: β-hydroxyethyltheophylline (10.9)

OTHER SUBSTANCES
Extracted: theophylline, paraxanthine, theobromine

KEY WORDS
serum; pharmacokinetics

REFERENCE
Oo,C.Y.; Burgio,D.E.; Kuhn,R.C.; Desai,N.; McNamara,P.J. Pharmacokinetics of caffeine and its metabolites in lactation: Prediction of milk to serum concentration ratios, *Pharm.Res.*, **1995**, *12*, 313–316.

SAMPLE
Matrix: blood, saliva
Sample preparation: Add 500 μL plasma, serum, or saliva to 200 mg ammonium sulfate, add 50 μL 15 μg/mL IS in water, add 500 μL 200 mM pH 4.5 sodium acetate buffer, vortex briefly, add 3 mL dichloromethane, shake at 85 cycles/min for 10 min, centrifuge at 2000 g for 10min. Evaporate the organic layer to dryness at 40° under a stream of nitrogen, reconstitute in 250 μL mobile phase, inject a 50 μL aliquot.

HPLC VARIABLES
Guard column: 20 × 2 pellicular C18
Column: 100 × 4.6 3 μm Microsorb MV C18
Mobile phase: MeOH:THF:100 mM pH 4.5 sodium acetate:water 6.5:1.4:10:82.1
Flow rate: 0.8
Injection volume: 50
Detector: UV 274

CHROMATOGRAM
Retention time: 9.1
Internal standard: β-hydroxyethyltheophylline (6.5)
Limit of detection: 5 ng/mL

OTHER SUBSTANCES
Extracted: metabolites, paraxanthine, theobromine, theophylline

Noninterfering: chlorzoxazone, dapsone

KEY WORDS
plasma; serum

REFERENCE
Frye,R.F.; Stiff,D.D.; Branch,R.A. A sensitive method for the simultaneous determination of caffeine and its dimethylxanthine metabolites in human plasma: Application to CYP1A2 phenotyping, *J.Liq.Chromatogr.Rel.Technol.*, **1998**, *21*, 1161–1171.

SAMPLE
Matrix: blood, saliva
Sample preparation: Saliva. Add 3 mg N-acetylcysteine (mucolytic agent) to 200 μL saliva), let stand for 10-15 min. 50 μL Saliva + 50 μL 1 M NaOH, extract with 1 mL 10% (v/v) isopropanol in chloroform (Caution! Chloroform is a carcinogen!) containing 1.5 mg/L β-hydroxyethyl theophylline. Centrifuge at 2000 g for 1 min. Evaporate organic layer to dryness under a gentle airflow at 60°. Dissolve residue in 200 μL mobile phase, inject a 50 μL aliquot. Serum. 50 μL Serum + 50 μL 1 M NaOH, extract with 1 mL 10% isopropanol in chloroform containing 1.5 mg/L β-hydroxyethyl theophylline. Centrifuge at 2000 g for 1 min. Evaporate organic layer to dryness under a gentle airflow at 60°. Dissolve the residue in 200 μL mobile phase, inject a 50 μL aliquot.

HPLC VARIABLES
Column: 150 × 3.9 C18 Nova Pak
Mobile phase: MeOH:THF:50 mM pH 4.7 ammonium acetate buffer 2:1.5:96.5
Flow rate: 1
Injection volume: 50

CHROMATOGRAM
Retention time: 10.2
Internal standard: β-hydroxyethyl theophylline (6.6)
Limit of quantitation: 200 ng/L

OTHER SUBSTANCES
Extracted: paraxanthine, theobromine, theophylline

KEY WORDS
serum

REFERENCE
Lee,T.C.; Charles,B.G.; Steer,P.A.; Flenady,V.J. Saliva as a valid alternative to serum in monitoring intravenous caffeine treatment for apnea of prematurity, *Ther.Drug Monit.*, **1996**, *18*, 288–293.

SAMPLE
Matrix: blood, tissue
Sample preparation: Tissue. Homogenize brain in twenty volumes of water, centrifuge at 1500 g, freeze at -20° for 2 h, centrifuge. 200 μL Supernatant + 100 μL pH 7.2 phosphate buffer, mix, add 6 mL dichloromethane:propanol 95:5, mix for 2 min, centrifuge at 1500 g for 10 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 35°, reconstitute the residue in 200 μL mobile phase, vortex for 1 min, inject a 25 μL aliquot. Serum. 200 μL Serum + 100 μL pH 7.2 phosphate buffer, mix, add 6 mL dichloromethane:propanol 95:5, mix for 2 min, centrifuge at 1500 g for 10 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 35°, reconstitute the residue in 200 μL mobile phase, vortex for 1 min, inject a 25 μL aliquot.

HPLC VARIABLES
Guard column: 10 μm μBondapak C18

Column: 250 × 4.6 10 μm μBondapak C18
Mobile phase: THF:10 mM Na_2HPO_4 3:97, pH adjusted to 6.5 with phosphoric acid
Flow rate: 2.5
Injection volume: 25
Detector: UV 273

CHROMATOGRAM
Retention time: 7
Limit of detection: 62.5 ng/mL (serum), 62.5 ng/g (tissue)

OTHER SUBSTANCES
Extracted: theophylline, theobromine, paraxanthine

KEY WORDS
serum; rat; brain

REFERENCE
Parra,P.; Limon,A.; Ferre,S.; Guix,T.; Jane,F. High-performance liquid chromatographic separation of caffeine, theophylline, theobromine and paraxanthine in rat brain and serum, *J.Chromatogr.*, **1991**, *570*, 185–190.

SAMPLE
Matrix: blood, urine
Sample preparation: Urine. 100 μL Urine + 100 μL 1 M NaOH, let stand at room temperature for 10 min, add 100 μL 1 M HCl, make up to 1 mL with mobile phase, vortex, centrifuge at 13000 g for 2 min, inject a 20 μL aliquot. Plasma. 500 μL Plasma + 100 μL 60% perchloric acid, add 200 μL 1 M NaOH to make pH >9, let stand for 10 min, add 200 μL 1 M HCl, add 200 μL 50 μg/mL β-hydroxyethyltheophylline, add 6 mL chloroform: isopropanol 50:50, agitate for 15 min on a rotating mixer, centrifuge at 4000 g. Remove the organic layer and evaporate it to dryness under vacuum, reconstitute the residue in 100 μL mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 100 × 4.5 5 μm ChromSep (Chrompack)
Mobile phase: THF:10 mM sodium acetate 0.8:99.2
Flow rate: 1
Injection volume: 20
Detector: UV 280

CHROMATOGRAM
Retention time: 7.03
Internal standard: β-hydroxyethyltheophylline (4.76)
Limit of quantitation: 1000 ng/mL

OTHER SUBSTANCES
Extracted: 1,3,7-trimethyluric acid, 1,7-dimethylxanthine, 1,3-dimethylxanthine, metabolites

KEY WORDS
plasma

REFERENCE
Dobrocky,P.; Bennett,P.N.; Notarianni,L.J. Rapid method for the routine determination of caffeine and its metabolites by high-performance liquid chromatography, *J.Chromatogr.B*, **1994**, *652*, 104–108.

SAMPLE
Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 205.2

CHROMATOGRAM
Retention time: 6.647

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: bulk
Sample preparation: Prepare a 750 μg/mL solution in 10 mM pH 2.5 orthophosphoric acid, sonicate for 10 min, filter (0.2 μm), inject a 15 μL aliquot.

HPLC VARIABLES
Guard column: 4 × 4 5 μm LiChrospher 100
Column: 125 × 4 3 μm Spherisorb ODS-1
Mobile phase: Gradient. A was water containing 5 mL/L 85% orthophosphoric acid and 0.56 mL/L hexylamine. B was MeCN:water 90:10 containing 5 mL/L 85% orthophosphoric acid and 0.56 mL/L hexylamine. A:B from 91:9 to 86:14 over 4 min, maintain at 86:14 for 13 min, to 55:45 over 11 min, maintain at 55:45 for 8 min, re-equilibrate at initial conditions for 20 min.
Flow rate: 0.7
Injection volume: 15
Detector: UV 210

CHROMATOGRAM
Retention time: 14.3

OTHER SUBSTANCES
Simultaneous: acetaminophen, acetylcodeine, benzocaine, cocaine, codeine, diamorphine, lidocaine, 6-monoacetylmorphine, morphine, noscapine, papaverine, procaine

REFERENCE

Grogg-Sulser,K.; Helmlin,H.-J.; Clerc,J.-T. Qualitative and quantitative determination of illicit heroin street samples by reversed-phase high-performance liquid chromatography: method development by CARTAGO-S, *J.Chromatogr.A*, **1995**, *692*, 121–129.

SAMPLE

Matrix: dialysate

Sample preparation: Dialyze blood with Ringer's solution, inject a 0.5 μL aliquot of the dialysate.

HPLC VARIABLES

Column: 14 × 1 3 μm BAS Sep-Stik ODS (Bioanalytical Systems)
Mobile phase: MeCN:50 mM pH 2.5 ammonium phosphate buffer 5:95
Flow rate: 0.2
Injection volume: 0.5
Detector: UV 273

CHROMATOGRAM

Retention time: 0.77
Limit of quantitation: 300 ng/mL

OTHER SUBSTANCES

Extracted: metabolites, theobromine, paraxanthine

KEY WORDS

rat; microbore

REFERENCE

Chen,A.; Lunte,C.E. Microdialysis sampling coupled on-line to fast microbore liquid chromatography, *J.Chromatogr.A*, **1995**, *691*, 29–35.

SAMPLE

Matrix: food

Sample preparation: Dilute 5 mL beverage with 5 mL mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 Partisil PXS 5/25 ODS-3 or 150 × 4.6 5μm Ultrasphere ODS
Mobile phase: MeOH:water:33% acetic acid 50:49:1
Injection volume: 20
Detector: UV 254

CHROMATOGRAM

Retention time: 5
Internal standard: aspirin (6.6)

KEY WORDS

drink; beverage; soft drink; tea; coffee

REFERENCE

Galasko,G.T.F.; Furman,K.I.; Alberts,E. The caffeine content of non-alcoholic beverages, *Food Chem.Toxicol.*, **1989**, *27*, 49–51.

SAMPLE

Matrix: formulations

Sample preparation: Powder tablets or capsules, weigh 20 mg, add 100 (tablets) or 500 (capsules) mL 50 mM sodium dodecyl sulfate, sonicate for 3 min, filter (sintered glass 4), dilute to a concentration of ca. 6 μg/mL with 50 mM sodium dodecyl sulfate, inject an aliquot.

HPLC VARIABLES
Guard column: 35 × 4.6 5 μm Spherisorb ODS-2 C18
Column: 120 × 4.5 μm Spherisorb ODS-2 C18
Mobile phase: Isopropanol:50 mM sodium dodecyl sulfate 1.5:98.5, pH 7.0
Flow rate: 1
Injection volume: 20
Detector: UV 273

CHROMATOGRAM
Retention time: 11.5

KEY WORDS
tablets; capsules

REFERENCE
Perez-Martinez,I.; Sagrado,S.; Medina-Hernández,M.J. Chromatographic determination of caffeine in pharmaceutical formulations using micellar mobile phases, *Chromatographia*, **1996**, *43*, 149–152.

SAMPLE
Matrix: formulations
Sample preparation: Add 50 mL of mobile phase to 0.5 g of sample and swirl to aid dissolution. Dilute to 100 mL with mobile phase. Dilute 1:10, filter (0.22 μm nylon). Inject a 10 μL aliquot.

HPLC VARIABLES
Column: 100 × 2.1 5 μm Hypersil ODS
Mobile phase: MeCN:water:triethylamine:acetic acid 5.5:94.1:0.2:0.2
Flow rate: 1.5
Injection volume: 10
Detector: UV 254

CHROMATOGRAM
Retention time: 2.2
Limit of quantitation: 5 μg/mL

OTHER SUBSTANCES
Simultaneous: acetaminophen, aspirin

KEY WORDS
powder

REFERENCE
Ferguson,G.K. Quantitative HPLC analysis of an analgesic/caffeine formulation: Determination of caffeine, *J.Chem.Educ.*, **1998**, *75*, 467–469.

SAMPLE
Matrix: formulations
Sample preparation: Weigh 500 mg homogenized analgesic powder, transfer to 100 mL volumetric flask, add ca. 50 mL mobile phase, swirl and dilute to volume with mobile phase. Dilute an aliquot of this solution 1:10 with mobile phase, filter (0.20 μm Cameo nylon filter, MSI, Westboro, MA) an aliquot, inject an aliquot of the filtrate.

HPLC VARIABLES
Column: 100 × 2.1 5 μm Hypersil ODS
Mobile phase: MeCN:triethylamine:acetic acid:water 5.5:0.2:0.2:94.1 (Prepare mobile phase as follows. Mix 110 mL MeCN, 4 mL triethylamine, 4 mL glacial acetic acid and make up to 2 L with water.)
Flow rate: 1.5
Injection volume: 10
Detector: UV 254

CHROMATOGRAM
Retention time: 2.2

OTHER SUBSTANCES
Extracted: aspirin, acetaminophen
Noninterfering: salicylic acid

KEY WORDS
powder

REFERENCE
Ferguson,G.K. Quantitative HPLC analysis of an analgesic/caffeine formulation: Determination of caffeine, *J.Chem.Educ.*, **1998**, *75*, 467–469.

SAMPLE
Matrix: formulations
Sample preparation: Sonicate 75 mg powdered tablets with 25 mL mobile phase for 15 min, filter (paper), inject a 135 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Ultrabase C18 (Scharlau Science, Spain)
Mobile phase: MeOH:20 mM pH 4.0 KH_2PO_4 30:70 adjusted to pH 4.0 with orthophosphoric acid
Flow rate: 1.5
Injection volume: 135
Detector: UV 274

CHROMATOGRAM
Retention time: 6.6
Limit of quantitation: 2.2 μg/mL

OTHER SUBSTANCES
Simultaneous: aspirin, salicylic acid, thiamine

KEY WORDS
tablets

REFERENCE
Gámiz-Gracia,L.; Luque de Castro,M.D. An HPLC method for the determination of vitamin B1, caffeine, acetylsalicylic acid, and the impurities of salicylic acid in a pharmaceutical preparation, *J.Liq.Chromatogr.Rel.Technol.*, **1997**, *20*, 2123–2133.

SAMPLE
Matrix: formulations
Sample preparation: Oils. 1 mL Sample + 25 mL MeOH:water 90:10, shake vigorously for 5 min, centrifuge, inject a 10 μL aliquot of the supernatant. Tablets. Grind a tablet to a fine powder, add 25 mL MeOH, sonicate for 5-10 min, filter (0.45 μm), discard first 5 mL of filtrate, inject a 10 μL aliquot of the remaining filtrate. Suspensions (aqueous).

Make up 5 mL to 50 mL with MeOH, filter (0.45 μm), discard first 5 mL of filtrate, inject a 10 μL aliquot of the remaining filtrate.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Zorbax ODS
Mobile phase: MeOH:water 75:25
Flow rate: 1.5
Injection volume: 10
Detector: UV 240

CHROMATOGRAM
Retention time: 2.6
Limit of detection: 5 μg/mL

OTHER SUBSTANCES
Simultaneous: benzyl alcohol, cortisone, fluoxymesterone, norethindrone, oxandrolone (UV 210), boldenone, ethisterone, methandrostenolone, nandrolone, norgestrel, testosterone, dehydroepiandrosterone (UV 210), mibolerone, methyltestosterone, methandriol (UV 210), norethindrone acetate, calusterone, mesterolone (UV 210), norethandrolone, trenbolone acetate, benzyl benzoate, nandrolone acetate, testosterone acetate, stanozolol, oxymetholone, nandrolone propionate, methenolone acetate, testosterone propionate
Interfering: aspirin, formebolone, testolactone

KEY WORDS
oils; tablets; suspensions

REFERENCE
Walters,M.J.; Ayers,R.J.; Brown,D.J. Analysis of illegally distributed anabolic steroid products by liquid chromatography with identity confirmation by mass spectrometry or infrared spectrophotometry, *J.Assoc.Off.Anal.Chem.*, **1990**, *73*, 904–926.

SAMPLE
Matrix: formulations

HPLC VARIABLES
Column: 100 × 4 3 μm Hypersil BDS-C18
Mobile phase: Gradient. MeCN:water adjusted to pH 2.1 from 0.3:99.7 to 25:75 over 11 min
Flow rate: 0.5
Detector: UV 220

CHROMATOGRAM
Retention time: 9

OTHER SUBSTANCES
Simultaneous: biotin, citric acid, folic acid, niacinamide, niacin, pantothenic acid, riboflavin, saccharin, thiamine, pyridoxine, vitamin B12, ascorbic acid

KEY WORDS
tablets

REFERENCE
Hewlett Packard Leaflet 12-5091-7351 EUS, 1993, **1993**,

SAMPLE
Matrix: formulations

Sample preparation: Powder tablet and add 50 mg to 50 mL MeCN:20 mM pH 3.8 phosphate buffer 3:97, sonicate for 5 min, filter (0.5 μm), inject a 20 μL aliquot of the filtrate.

HPLC VARIABLES
Guard column: Supelguard pre-column containing 5 μm Suplex pKb100 (Supelco)
Column: 150 × 4.6 5 μm Suplex pKb100 (Supelco)
Mobile phase: Gradient. MeCN:20 mM pH 3.8 phosphate buffer at 3:97 for 3 min, to 15:85 over 5 min, stay at 15:85 for 4 min, re-equilibrate for 8 min.
Flow rate: 1.5
Injection volume: 20
Detector: UV 220 for 5 min then UV 280

CHROMATOGRAM
Retention time: 10.40
Limit of quantitation: 3 μg/mL

OTHER SUBSTANCES
Simultaneous: methamphetamine, amphetamine, ephedrine, 3,4-methylenedioxyamphetamine, N-methyl-3,4-methylenedioxyamphetamine, N-ethyl-3,4-methylenedioxyamphetamine

KEY WORDS
tablets

REFERENCE
Longo,M.; Martines,C.; Rolandi,L.; Cavallaro,A. Simple and fast determination of some phenethylamines in illicit tablets by base-activated reversed phase HPLC, *J.Liq.Chromatogr.*, **1994**, *17*, 649–658.

SAMPLE
Matrix: formulations
Sample preparation: Finely powder half a tablet, add 9 mL mobile phase, sonicate for 20 min, make up to 10 mL with mobile phase, filter (Whatman type 40 and 0.2 μm Millipore), inject an aliquot of the filtrate.

HPLC VARIABLES
Column: 250 × 4 5 μm LiChrospher 100 CN
Mobile phase: MeCN:THF:buffer 7:6:87 (Buffer was 0.8% acetic acid containing 5 mM sodium hexanesulfonate, 10 mM di-n-butylamine, and 0.12% phosphoric acid, pH 3.3.)
Flow rate: 1
Injection volume: 20
Detector: UV 298

CHROMATOGRAM
Retention time: 3.8
Limit of detection: 1.1 μg/mL

OTHER SUBSTANCES
Simultaneous: acetaminophen (UV 310), chlorpheniramine (UV 265), guaifenesin (glycerylguaicolate) (UV 284), phenylpropanolamine (UV 260)

KEY WORDS
tablets

REFERENCE
Indrayanto,G.; Sunarto,A.; Adriani,Y. Simultaneous assay of phenylpropanolamine hydrochloride, caffeine, paracetamol, glycerylguaiacolate and chlorpheniramine in Silabat™ tablet using HPLC with diode array detection, *J.Pharm.Biomed.Anal.*, **1995**, *13*, 1555–1559.

SAMPLE
Matrix: formulations
Sample preparation: Finely powder tablets, weigh out amount equivalent to 20 mg enalapril maleate, suspend in 100 mL mobile phase, filter, inject a 5 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 12 μm Hypersil C18
Mobile phase: MeCN:water 20:80 adjusted to pH 3.8 with acetic acid
Flow rate: 1
Injection volume: 5
Detector: UV 215 for 3.5 min, then UV 275

CHROMATOGRAM
Retention time: 4.8

OTHER SUBSTANCES
Simultaneous: enalapril, hydrochlorothiazide

KEY WORDS
tablets

REFERENCE
el Walily,A.F.M.; Belal,S.F.; Heaba,E.A.; El Kersh,A. Simultaneous determination of enalapril maleate and hydrochlorothiazide by first-derivative ultraviolet spectrophotometry and high-performance liquid chromatography, *J.Pharm.Biomed.Anal.*, **1995**, *13*, 851–856.

SAMPLE
Matrix: formulations
Sample preparation: Weigh out powdered sample containing 8 mg caffeine, add 80 mL MeOH, sonicate for 10 min, dilute to 100 mL with MeOH, centrifuge. Remove a 5 mL aliquot of the supernatant and add it to 1 mL 2 mg/mL resorcinol, add 2 mL MeOH, make up to 20 mL with 50 mM pH 3.0 triethylamine phosphate, inject an aliquot.

HPLC VARIABLES
Column: 150 × 3.2 5 μm Hypersil ODS
Mobile phase: THF:50 mM pH 3.0 triethylamine phosphate 12:88
Flow rate: 0.6
Injection volume: 20
Detector: UV 275

CHROMATOGRAM
Retention time: 15
Internal standard: resorcinol (9)

OTHER SUBSTANCES
Simultaneous: acetaminophen, aspirin (post-column irradiation gives an increase in peak height), propyphenazone

REFERENCE
Di Pietra,A.M.; Gatti,R.; Andrisano,V.; Cavrini,V. Application of high-performance liquid chromatography with diode-array detection and on-line post-column photochemical derivatization to the determination of analgesics, *J.Chromatogr.A*, **1996**, *729*, 355–361.

SAMPLE
Matrix: microsomal incubations
Sample preparation: 1 mL Microsomal incubation + 1 mL chloroform:isopropanol 85:15 (Caution! Chloroform is a carcinogen!). Add 25 pmole 4-acetoimidophenol. (Metabolites

were extracted with the addition of an additional 3.5 mL chloroform:isopropanol 85:15 and 500 mg ammonium sulfate before centrifugation.). Mix, centrifuge at 3000 rpm for 10 min. Remove the organic phase, evaporate it and reconstitute the residue with 150 μL mobile phase A. Inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Beckman C18 (Phenomenex, USA)
Mobile phase: Gradient. A was THF:50 mM pH 4.5 sodium acetate 1:99. B was MeCN:50 mM pH 4.5 sodium acetate 60:40. A:B 100:0 for 1 min, to 70:30 over 23 min, to 0:100 over 1 min, maintain at 0:100 for 5 min, to 100:0 over 1 min, maintain at 100:0 for 9 min.
Flow rate: 0.8
Detector: UV 270

CHROMATOGRAM
Retention time: 18
Internal standard: 4-acetoimidophenol (16)

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
liver

REFERENCE
Ring,B.J.; Catlow,J.; Lindsay,T.J.; Gillespie,T.; Roskos,L.K.; Cerimele,B.J.; Swanson,S.P.; Hamman,M.A.; Wrighton,S.A. Identification of the human cytochromes P450 responsible for the in vitro formation of the major oxidative metabolites of the antipsychotic agent olanzapine, *J.Pharmacol.Exp.Ther.*, **1996**, *276*, 658–666.

SAMPLE
Matrix: microsomal incubations
Sample preparation: 275 μL Microsomal incubation + 50 μL 30% perchloric acid, centrifuge at 2000 g for 10 min, inject a 60 μL aliquot of the supernatant.

HPLC VARIABLES
Guard column: 20 × 4.6 40 μm Supelcosil LC-18
Column: 150 × 4.6 5 μm Supelcosil LC-18
Mobile phase: MeCN:THF:20 mM pH 3.5 sodium perchlorate 0.5:0.5:99
Flow rate: 1.5
Injection volume: 60
Detector: UV 280

CHROMATOGRAM
Retention time: 35

OTHER SUBSTANCES
Extracted: paraxanthine, theobromine, theophylline, trimethylurate

KEY WORDS
monkey; liver

REFERENCE
Bullock,P.; Pearce,R.; Draper,A.; Podval,J.; Bracken,W.; Veltman,J.; Thomas,P.; Parkinson,A. Induction of liver microsomal cytochrome P450 in cynomolgus monkeys, *Drug Metab.Dispos.*, **1995**, *23*, 736–748.

SAMPLE
Matrix: milk
Sample preparation: Centrifuge at 12800 g for 10 min, remove top layer of fat with a spatula. 450 μL Supernatant + 50 μL water + 150 μL 21.65 μg/mL proxyphylline in 6% perchloric acid, vortex for 5 s, cool on ice for 10-15 min, centrifuge at 12800 g for 10 min, inject a 50 μL aliquot of the supernatant

HPLC VARIABLES
Guard column: Co:Pell ODS glass beads (Whatman)
Column: 250 × 4.6 5 μm Ultrasphere ODS
Mobile phase: Gradient. A was 10 mM sodium acetate + 5 mM tetrabutylammonium hydrogen sulfate, adjusted to pH 4.9 with 2 M NaOH. B was MeOH:water 50:50 containing 10 mM sodium acetate + 5 mM tetrabutylammonium hydrogen sulfate, adjusted to pH 4.8 with glacial acetic acid. A:B 100:0 for 7.5 min, then to 85:15 over 7.5 min, then to 70:30 over 10 min, then to 68:32 over 4 min, then to 100:0 over 3 min
Flow rate: 1.5
Injection volume: 50
Detector: UV 272

CHROMATOGRAM
Retention time: 30
Internal standard: proxyphylline (25)

OTHER SUBSTANCES
Simultaneous: theobromine, paraxanthine, theophylline

REFERENCE
Blanchard,J.; Weber,C.W.; Shearer,L.E. HPLC analysis of methylxanthines in human breast milk, *J.Chromatogr.Sci.*, **1990**, *28*, 640–642.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4 ODS (Hitachi)
Mobile phase: MeCN:50 mM phosphoric acid 18:82
Column temperature: 55
Flow rate: 0.6
Injection volume: 20
Detector: UV 273

REFERENCE
Sugawara,M.; Takekuma,Y; Yamada,H.; Kobayashi,M.; Iseki,K.; Miyazaki,K. A general approach for the prediction of the intestinal absorption of drugs: regression analysis using the physicochemical properties and drug-membrane electrostatic interactions, *J.Pharm.Sci.*, **1998**, *87*, 960–966.

SAMPLE
Matrix: solutions
Sample preparation: Dissolve in MeOH at a concentration of 1 mg/mL, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 250 × 5 Spherisorb S5W
Mobile phase: MeOH:buffer 90:10 (Buffer was 94 mL 35% ammonia and 21.5 mL 70% nitric acid in 884 mL water, adjust the pH to 10.1 with ammonia.)
Flow rate: 2
Injection volume: 20

Detector: UV 254

CHROMATOGRAM
Retention time: 1.66

OTHER SUBSTANCES
Simultaneous: levallorphan, hydroxypethidine, normethadone, meperidine, dipipanone, diamorphine, pentazocine, acetylcodeine, monoacetylmorphine, thebacon, oxycodone, thebaine, norlevorphanol, methadone, benzylmorphine, ethylmorphine, morphine-N-oxide, codeine, codeine-N-oxide, morphine, ethoheptazine, morphine-3-glucuronide, pholcodeine, norpethidine, hydrocodone, dihydrocodeine, dihydromorphine, levorphanol, norcodeine, normorphine, epinephrine, pipradol, phenylpropanolamine, fencamfamin, chlorphentermine, norpseudoephedrine, phentermine, fenfluramine, methylenedioxyamphetamine, amphetamine, normetanephrine, 4-hydroxyamphetamine, bromo-STP, STP, prolintane, 2-phenethylamine, tyramine, trimethoxyamphetamine, phenylephrine, pseudoephedrine, ephedrine, methylephedrine, dimethylamphetamine, methamphetamine, mescaline, mephentermine, buprenorphine, dextromoramide, phenoperidine
Noninterfering: dopamine, levodopa, methyldopa, methyldopate, norepinephrine
Interfering: pemoline, benzphetamine, diethylpropion, mazindol, tranylcypromine, fenethyline, phendimetrazine, methylphenidate, phenelzine, fentanyl, etorphine, piritramide, noscapine, papaverine, naloxone, dextropropoxyphene, nalorphine, phenazocine, norpipanone

REFERENCE
Law,B.; Gill,R.; Moffat,A.C. High-performance liquid chromatography retention data for 84 basic drugs of forensic interest on a silica column using an aqueous methanol eluent, *J.Chromatogr.*, **1984**, *301*, 165–172.

SAMPLE
Matrix: solutions
Sample preparation: Dissolve compounds in MeCN:water 80:20, inject a 1 μL aliquot.

HPLC VARIABLES
Column: 150 × 1 3 μm Hitachi-Gel 3057 ODS silica (Hitachi)
Mobile phase: MeCN:water 25:75
Flow rate: 0.03
Injection volume: 1
Detector: UV 254

CHROMATOGRAM
Retention time: 5

OTHER SUBSTANCES
Simultaneous: dipyrone (sulpyrin), guaifenesin (guaiacol glycerol ether), acetaminophen, bucetin (3-hydroxy-p-butyrophenetidine), methyl p-hydroxybenzoate, phenacetin

KEY WORDS
semi-micro

REFERENCE
Matsushima,Y.; Nagata,Y.; Niyomura,M.; Takakusagi,K.; Takai,N. Analysis of antipyretics by semimicro liquid chromatography, *J.Chromatogr.*, **1985**, *332*, 269–273.

SAMPLE
Matrix: solutions
Sample preparation: Dissolve in MeOH:water 1:1 at a concentration of 50 μg/mL, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeOH:acetic acid:triethylamine:water 25:1.5:0.5:73
Flow rate: 1.5
Injection volume: 10
Detector: UV 254

CHROMATOGRAM
Retention time: 11

OTHER SUBSTANCES
Simultaneous: theobromine, diphylline, theophylline, 8-chlorotheophylline

REFERENCE
Roos,R.W.; Lau-Cam,C.A. General reversed-phase high-performance liquid chromatographic method for the separation of drugs using triethylamine as a competing base, *J.Chromatogr.*, **1986**, *370*, 403–418.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 150 × 4.6 Supelcosil LC-ABZ
Mobile phase: MeCN:25 mM pH 6.9 potassium phosphate buffer 35:65
Flow rate: 1.5
Injection volume: 25
Detector: UV 254

CHROMATOGRAM
Retention time: 1.329

OTHER SUBSTANCES
Also analyzed: 6-acetylmorphine, amiloride, amphetamine, benzocaine, benzoylecgonine, cocaine, codeine, doxylamine, fluoxetine, glutethimide, hexobarbital, hypoxanthine, levorphanol, LSD, meperidine, mephobarbital, methadone, methylphenidate, methyprylon, N-norcodeine, oxazepam, oxycodone, phenylpropanolamine, prilocaine, procaine, terfenadine

REFERENCE
Ascah,T.L. Improved separations of alkaloid drugs and other substances of abuse using Supelcosil LC-ABZ column, *Supelco Reporter*, **1993**, *12(3)*, 18–21.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Guard column: 30 × 3.2 7 μm SI 100 ODS (not commercially available)
Column: 150 × 3.2 7 μm SI 100 ODS (not commercially available)
Mobile phase: MeCN:buffer 31.2:68.8 (Buffer was 6.66 g KH_2PO_4 and 4.8 g 85% phosphoric acid in 1 L water, pH 2.3.)
Flow rate: 0.5-1
Detector: UV 225, 267

CHROMATOGRAM
Retention time: 1.5
Internal standard: 5-(4-methylphenyl)-5-phenylhydantoin (7.3)

OTHER SUBSTANCES

Also analyzed: aspirin, carbamazepine, chlordiazepoxide, chlorprothixene, clonazepam, diazepam, doxylamine, ethosuximide, furosemide, haloperidol, hydrochlorothiazide, methocarbamol, methotrimeprazine, nicotine, oxazepam, procaine, promazine, propafenone, propranolol, salicylamide, temazepam, tetracaine, thiopental, triamterene, verapamil, zolpidem, zopiclone

REFERENCE

Below,E.; Burrmann,M. Application of HPLC equipment with rapid scan detection to the identification of drugs in toxicological analysis, *J.Liq.Chromatogr.*, **1994**, *17*, 4131–4144.

SAMPLE

Matrix: solutions

Sample preparation: Dilute a 10 mg/mL solution of caffeine in water 1:1000 with mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 2540 × 4.6 5 μm C18 (Supelco)

Mobile phase: MeOH:10 mM ammonium acetate and 2.5 mM sodium heptanesulfonate 20:80, pH adjusted to 5.1 with glacial acetic acid

Flow rate: 2

Injection volume: 20

Detector: UV 254

CHROMATOGRAM

Retention time: 10.5

OTHER SUBSTANCES

Simultaneous: benzoic acid

KEY WORDS

stability-indicating; water

REFERENCE

Donnelly,R.F.; Tirona,R.G. Stability of citrated caffeine injectable solution in glass vials, *Am.J.Hosp.Pharm.*, **1994**, *51*, 512–514.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 Zorbax RX

Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.

Column temperature: 30

Flow rate: 2

Detector: UV 210

OTHER SUBSTANCES

Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheni-

ramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 5 μm Supelcosil LC-DP (A) or 250 × 4 5 μm LiChrospher 100 RP-8 (B)
Mobile phase: MeCN:0.025% phosphoric acid:buffer 25:10:5 (A) or 60:25:15 (B) (Buffer was 9 mL concentrated phosphoric acid and 10 mL triethylamine in 900 mL water, adjust pH to 3.4 with dilute phosphoric acid, make up to 1 L.)
Flow rate: 0.6
Injection volume: 25
Detector: UV 229

CHROMATOGRAM

Retention time: 4.79 (A), 3.84 (B)

OTHER SUBSTANCES

Also analyzed: acebutolol, acepromazine, acetaminophen, acetazolamide, acetophenazine, albuterol, alprazolam, amitriptyline, amobarbital, amoxapine, antipyrine, atenolol, atropine, azatadine, baclofen, benzocaine, bromocriptine, brompheniramine, brotizolam, bupivacaine, buspirone, butabarbital, butalbital, carbamazepine, cetirizine, chlorcyclizine, chlordiazepoxide, chlormezanone, chloroquine, chlorpheniramine, chlorpromazine, chlorpropamide, chlorprothixene, chlorthalidone, chlorzoxazone, cimetidine, cisapride, clomipramine, clonazepam, clonidine, clozapine, cocaine, codeine, colchicine, cyclizine, cyclobenzaprine, dantrolene, desipramine, diazepam, diclofenac, diflunisal, diltiazem, diphenhydramine, diphenidol, diphenoxylate, dipyridamole, disopyramide, dobutamine, doxapram, doxepin, droperidol, encainide, ethidium bromide, ethopropazine, fenoprofen, fentanyl, flavoxate, fluoxetine, fluphenazine, flurazepam, flurbiprofen, fluvoxamine, furosemide, glutethimide, glyburide, guaifenesin, haloperidol, homatropine, hydralazine, hydrochlorothiazide, hydrocodone, hydromorphone, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, ketoconazole, ketoprofen, ketorolac, labetalol, levorphanol, lidocaine, loratadine, lorazepam, lovastatin, loxapine, mazindol, mefenamic acid, meperidine, mephenytoin, mepivacaine, mesoridazine, metaproterenol, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyldopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymetazoline, paroxetine, pemoline, pentazocine, pentobarbital, pentoxifylline, perphenazine, pheniramine, phenobarbital, phenol, phenolphthalein, phentolamine, phenylbutazone, phenyltoloxamine, phenytoin, pimozide, pindolol, piroxicam, pramoxine, prazepam, prazosin, probenecid, procainamide, procaine, prochlorperazine, procyclidine, promazine, promethazine, propafenone, propantheline, propiomazine, propofol, propranolol, protriptyline, quazepam, quinidine, quinine, racemethorphan, ranitidine, remoxipride, risperidone, salicylic acid, scopolamine, secobarbital, sertraline, sotalol, spironolactone, sulfinpyrazone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocainide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

KEY WORDS

also details of plasma extraction

REFERENCE

Koves,E.M. Use of high-performance liquid chromatography-diode array detection in forensic toxicology, *J.Chromatogr.A*, **1995**, *692*, 103–119.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 150 mm long 5 μm Microsorb-MV C18
Mobile phase: MeOH:water 60:40
Flow rate: 1
Detector: UV 270

REFERENCE

Phillips,C.A.; Michniak,B.B. Transdermal delivery of drugs with differing lipophilicities using azone analogs as dermal penetration enhancers, *J.Pharm.Sci.*, **1995**, *84*, 1427–1433.

SAMPLE

Matrix: urine
Sample preparation: 40 μL Urine + 100 μL 160 μM IS in 30% EtOH, make up to 400 μL with 10 mM pH 4.0 acetate buffer. Add 5 mL ethyl acetate:2-propanol 93:7, shake for 10

min, centrifuge at 1000 g for 10 min, freeze the aqueous phase at -30°. Evaporate organic phase to dryness under a stream of nitrogen at 55°. Reconstitute the residue in 300 μL mobile phase, vortex for 10 s, centrifuge at 1000 g for 30 s, inject a 40 μL aliquot. (Keep sample in the autosampler at 5°.)

HPLC VARIABLES
Column: 250 × 4.6 5 μm Ultrasphere ODS
Mobile phase: MeOH:10 mM pH 4.0 acetate buffer 9:91
Column temperature: 30
Flow rate: 1 from 0 to 11 min, 1.5 from 11 to 17 min, 2.5 from 17 to 30 min
Injection volume: 40
Detector: UV 273

CHROMATOGRAM
Retention time: 25
Internal standard: β-hydroxy-ethyl theophylline (19.8)

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
pharmacokinetics

REFERENCE
Rasmussen,B.B.; Brosen,K. Determination of urinary metabolites of caffeine for the assessment of cytochrome P4501A2, xanthine oxidase, and N-acetyltransferase activity in humans, *Ther.Drug Monit.*, **1996**, *18*, 254–262.

SAMPLE
Matrix: urine
Sample preparation: Add 100-120 mg NaCl, 50 μL 100 μg/mL β-hydroxyethyltheophylline, and 100 μL ammonia buffer to 1 mL urine. Extract with 5 mL MeOH:dichloromethane 10:90 for 10 min, centrifuge at 150 g for 5 min, remove the upper aqueous layer and evaporate the organic layer under nitrogen at 40°. Dissolve the residue in 200 μL mobile phase, inject a 20 μL aliquot. (Prepare the pH 9.5 ammonia buffer by adding ammonia to a saturated ammonium chloride solution.)

HPLC VARIABLES
Guard column: 10 × 2 40 μm C18
Column: 100 × 3 5 μm Hypersil 5 ODS (Chrompack)
Mobile phase: THF:water 1:100
Flow rate: 1
Injection volume: 20
Detector: UV 275

CHROMATOGRAM
Retention time: 6.5
Internal standard: β-hydroxyethyltheophylline (4.5)

OTHER SUBSTANCES
Extracted: theophylline, theobromine, paraxanthine

KEY WORDS
horse; human; urine

REFERENCE
Delbeke,F.T.; De Backer,P. Threshold level for theophylline in doping analysis, *J.Chromatogr.B*, **1996**, *687*, 247–252.